

EXHIBIT B

From Page No. _____

Synthesis of Cht A 176

5' > CGT, TAC, TCG, GAT, GCC, CAA, ATA, TCG, CCA, CAT, TCG < 3'

33 BASES TOTAL

Cht A 176

Conditions:

ABI 380A DNA Synthesizer

BCE Phosphoramidite (A from cracker freshly made, G, C, T from cracker made)

Cycle RB3MW

tr. tgl off, manual

G column, ABI, 1.0 umol (Yellow # 320848)

- 33 tritiated colors were collected
column dried \geq Argon gas for 15'

Total weight of resin = 41 mg
 $1/3 = 14$ mg deblocked at 55°C for 5 hours
 27 mg kept at 4°C in small vial

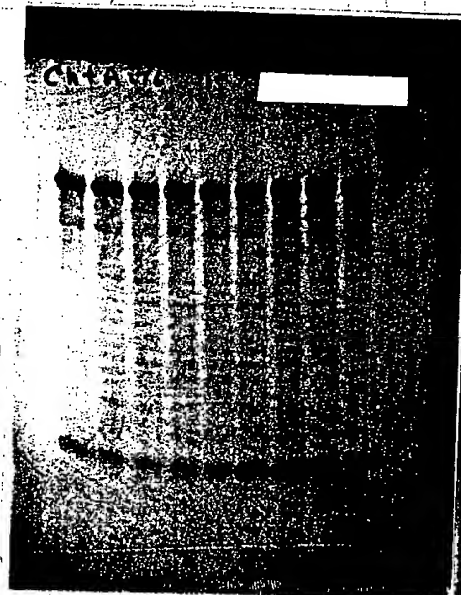
Total Crude O.D. = 69.5
 O.D. gel purified = 27.8 O.D. loaded on a 15%
 denaturing gel in 9 lanes

The slowest moving band was isolated, crushed &
 suspended in 0.1M NH_4OAc pH=7.0 O/N at
 Room Temp in wrist shaker.

Cht A 176 isolated using PD-10 column using Procedure (237-4). O.D. measured.

Total O.D. Purified = 5.76
 Concentration = 1.28 O.D./ml

Kinase

on Page 40 \rightarrow next Page

To Page No. 40

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 39

~~Wavelength measurement~~

Kination

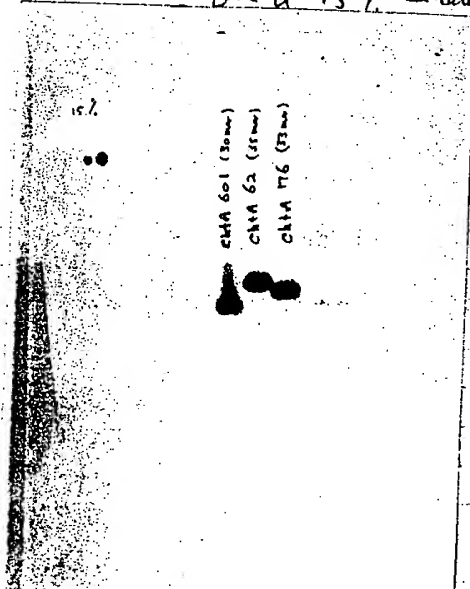
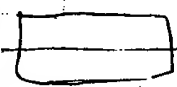
7.5 ul ChtA 176
 1.0 ul 10XKB
 1.0 ul γ - 32 P-ATP (G# 474)
 0.5 ul T₄ Kinal
 (Pharmacia N07344)
 Incubate at 37°C for 30'
 72°C for 2'
 Chill on ice
 Precipitate:
 200ul 5M NH₄OAc
 0.5 ul 0.5M EDTA
 600ul glycogen
 800ul ethanol
 Wash in 80% ethanol
 Dissolve in 10ul dH₂O
 Load 2ul (2x10⁵ cpm)
 on a 15% denaturing gel

As the picture of
 autoradiogram
 shows
 ChtA 176 is
 pure & has
 correct length
 of 33 bases
 5' = 12800
 was given
 to the
 requester.

ChtA 601 (3000)
 ChtA 62 (1500)
 ChtA 176 (3200)

Base — A498nm

6	G 3'	1.449	
7	C	1.474	100%
8	T	1.365	93%
9	T	1.358	99%
10	A	1.304	96%
11	C	1.245	95%
12	A	1.238	99%
13	C	1.187	96%
14	C	1.268	100%
15	G	1.226	97%
16	C	1.188	97%
17	T	1.198	100%
18	A	1.172	98%
19	T	1.103	94%
20	A	1.068	97%
21	A	1.057	99%
22	C	0.873	83% *
23	C	0.880	100%
24	C	0.867	98%
25	C	0.841	97%
26	G	0.951	>100%
27	T	0.870	91% -
28	A	0.842	97%
29	G	0.822	98%
30	G	0.8130	99%
31	C	0.8220	100%
32	T	0.788	96%
33	C	0.781	99%
34	A	0.774	99%
35	T	0.735	95%
36	T	0.733	100%
37	G	0.741	100%
38	C	0.723	97%



Witnessed & Understood by me.

Date

Invented by

Date

Recorded by

Page No. _____